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EXERCISE AND OXIDATIVE STRESS: SOURCES OF FREE RADICALS AND THEIR IMPACT ON ANTIOXIDANT SYSTEMS

Li Li Ji and **Steve Leichtweis**
Department of Kinesiology,
Interdepartmental Program of Nutritional Sciences, and Institute on Aging
University of Wisconsin-Madison

ABSTRACT

Strenuous exercise is characterized by increased oxygen consumption and the disturbance between intracellular pro-oxidant and antioxidant homeostasis. At lease three biochemical pathways (i.e., mitochondrial electron transport chain, xanthine oxidase, and polymorphoneutrophil) have been identified as potential sources of intracellular free radical generation during exercise. These deleterious reactive oxygen species pose a serious threat to the cellular antioxidant defense system, such as diminished reserves of antioxidant vitamins and glutathione. However, enzymatic and non-enzymatic antioxidants have demonstrated great versitility and adaptability in response to acute and chronic exercise. The delicate balance between pro-oxidants and antioxidants suggests that supplementation with antioxidants may be desirable for physically active individuals under certain physiological conditions by providing a larger protective margin.

KEY WORDS

Antioxidants, Exercise, Free radicals, Oxidative stress, Vitamins

INTRODUCTION

Except for strict anaerobes, most organisms utilize oxygen as an electron acceptor to oxidize the various metabolic substrates so that stored energy is released for biological activities. During this process, most oxygen molecules are reduced to water, but a fraction of oxygen (2-5%) is univalently reduced to various intermediates representing the electron reductants of oxygen: one, superoxide ($O_2^{\cdot-}$); two, hydrogen peroxide (H_2O_2), and three, hydroxyl radical ($^{\cdot}OH$) (1). These reactive oxygen species (ROS) have a strong tendency of extracting electrons to reach a chemically more stable structure and therefore are capable of eliciting serious damage to the various cellular components (2). Cells also utilize ROS to assist in the elimination of xenobiotic compounds and organisms through phagocytosis, which involves a respiratory burst and $O_2^{\cdot-}$ production (3). Although this process is generally considered beneficial to the cell, it could also indiscriminately subject the cell to oxidative damage. In addition, oxygen serves as an electron acceptor in the oxidation of D-amino acids in the peroxisome; in the activation of cytochrome P_{450} in the microsome; in the metabolism of xanthine and hypoxanthine; and in the auto-oxidation of catecholamines. All these processes are capable of producing ROS (4). It is estimated that a normal cell produces $2x10^{10}$ $O_2^{\cdot-}$ and H_2O_2 per day, which amounts to $3.3x10^{-14}$ moles per day (2). The constant contact and reaction of the cellular constituents, including genetic materials, with ROS is proposed to be a main mechanism of organism aging (5).

Aerobic organisms would not survive without protective mechanisms counteracting the detrimental effects of ROS. Thus, higher organisms have developed effective antioxidant systems during the course of evolution (1,6). In general, the cell has adequate antioxidant reserves to cope with the production of ROS under physiological conditions such that these toxic compounds do not accumulate. The system consists of antioxidant vitamins (water-soluble ascorbic acid and fat-soluble a-tocopherol and b-carotene), thiol-containing, low-molecular weight compounds, mainly glutathione (GSH), and antioxidant enzymes, such as superoxide dismutase (SOD), GSH peroxidase (GPX), and catalase (CAT). Each of these antioxidants plays a unique role in the cell and complements one another geographically and functionally (6). Furthermore, there is evidence that certain antioxidants, such as GSH, may be involved in interorgan transport (7). These antioxidant defense systems preserve homeostasis for normal cell function at rest and perhaps during mild oxidative stress. However, the protective margin of most antioxidants is probably very small. Therefore, when ROS production is excessive, or when the antioxidant defense is severely compromised due to nutritional deficiency or biochemical inhibition, extensive cell and tissue damage may occur, leading to various pathogenic conditions and/or aging (2). The resultant oxidative damage can induce further ROS production thereby forming a vicious cycle. In the past decade, evidence has accumulated showing that unaccustomed and strenuous exercise may manifest an imbalance between ROS and antioxidant defenses, resulting in an oxidatively stressful environment in the body. This disturbance of homeostasis is implied in numerous physiological disorders occurring during and after exercise, such as fatigue, muscle soreness, myofibril disruption,

To whom all correspondence should be addressed:
Li Li Ji, Ph.D.; 2000 Observatory Drive; Madison, WI 53706
Tel. (608) 262-7250 / Fax (608) 262-1656

and impairment of immune function (8). The purpose of this article is two-fold, (i) to review several major theories propagating the possible mechanisms by which exercise may lead to cellular oxidative stress and damage; and (ii) to summarize, based upon available evidence, the short-term and long-term strategies for cell protection against ROS-inflicted oxidative challenge.

FREE RADICAL GENERATION DURING EXERCISE

The implication that ROS may play an important role in exercise-induced tissue damage appeared in the literature in the late 1970s (9,10). It is now widely accepted that many of the disorders at the cell, tissue, or organ level observed either immediately after heavy exercise or post-exercise may be attributed to ROS generation. Several comprehensive reviews have been published in this area (8,11-14). However, the biochemical mechanism(s) by which ROS production is increased during exercise is still largely elusive. Several theories, whether or not explicitly stated, have been postulated to explain this observation. These theories are not mutually exclusive, and may apply to specific organs, tissues, and cellular locations under certain physiological conditions.

Mitochondrial Theory

The majority of oxygen consumed by the eukaryote cells are reduced in the mitochondria via the electron transport chain (ETC). Both NADH-ubiquinone reductase and ubiquinone-cytochrome c reductase generate $O_2^{\cdot-}$ and H_2O_2 (6). Because the transition from a two-electron (NADH and $FADH_2$) to a one-electron (ubiquinone) transfer involves the formation of semiubiquinone ($QH^{\cdot}$), this segment of the ETC becomes a primary site for $O_2^{\cdot-}$ production (6) . $O_2^{\cdot-}$ is readily reduced to H_2O_2 by mitochondrial SOD (manganese-containing). A metal-catalyzed Fenton reaction or Haber-Weiss reaction between $O_2^{\cdot-}$ and H_2O_2 may give rise to $^{\cdot}OH$ (1). Liver mitochondria is estimated to produce 24 nmol $O_2^{\cdot-}$ min per g of tissue; with an active conversion to H_2O_2 by SOD, a steady-state $O_2^{\cdot-}$ concentration of $8x10^{-8}$ M can be derived (6). Heart mitochondria generate 0.3-0.6 nmol/min per mg protein representing 2% of the tissue's total oxygen consumption. Mitochondrial H_2O_2 production has shown to increase with increased O_2 tension in the environment (6).

The premise that exercise increases mitochondrial ROS production is based on the well-known fact that tissue and whole body oxygen consumption is increased dramatically during strenuous exercise. During maximal exercise, whole body oxygen consumption (VO_2) increases up to 20-fold, while VO_2 at the muscle fiber level is estimated to be elevated by as much as 100-fold (8). Assuming that the percentage of O_2 to become $O_2^{\cdot-}$ remains the same (i.e., ETC efficiency maintains the same), ROS production will increase roughly proportionally. However, evidence shows that heavy exercise may induce mitochondrial uncoupling due to inner membrane damage and hyperthermia (15,16). Thus, the actual rate of ROS production may be higher than that calculated from basal ROS production.

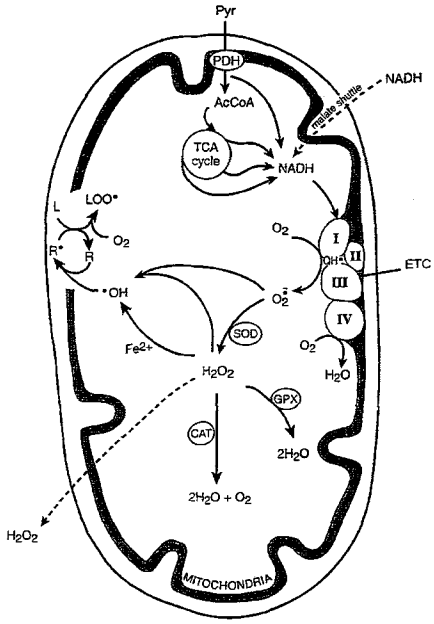

Fig. 1: Generation of reactive oxygen species in the mitochondria. CAT, catalase; ETC, electron transport chain; GPX, glutathione peroxidase; LOO• lipid peroxy radical; PDH, pyruvate dehydrogenase complex; QH , semiquinone; R , alkyl radical; SOD, superoxide dismutase. Reactions are not balanced stoichiometrically.

There is no direct demonstration that mitochondrial $O_2^{\cdot-}$ production is increased during exercise. Using electron paramagnetic resonance (EPR) method, Davies et al. (15) showed that free radical signals recorded in muscle and liver homogenate from exercised rats are increased significantly compared to the rested controls. Since tissues were taken immediately from exhaustively exercised rats and the free radicals were identified as semiquinone (g = 2.004), the origin of the free radicals detected in the study was presumably mitochondria. Although several other authors also reported increased free radical generation in various tissue samples from exercised animals in vivo (17,18) and in vitro (19), the source of the free radicals were not identified. In an isolated diaphragm preparation, Reid et al. (20) demonstrated the oxidation of dichlorofluoroscin (DCF) in the muscle when the contraction was at low frequency. SOD was found to attenuate the level of DCF formation, indicating that a major source of oxidants might be $O_2^{\cdot-}$. The fact that ROS were observed when the muscle was stimulated to contract at low frequency suggests that the ROS was of mitochondrial origin.

The hypothesis that mitochondria are a primary site of ROS generation during exercise is supported by numerous studies. State 4 respiration is shown to be increased in muscle and liver (15) and heart mitochondria (21) after exhaustive exercise, indicating a possible inner member leakage inflicted by ROS. In these studies, the

respiratory control index was decreased mainly as a result of the augmented state 4 respiration, with no change or a proportionally smaller increase in state 3 respiration in response to exercise. These changes coincide with enhanced mitochondrial lipid peroxidation (15), and are consistent with the findings that an acute bout of exercise could lead to loss of mitochondrial protein thiol content and inactivation of oxidative enzymes (22). Furthermore, both muscle and heart mitochondria from animals involved in high-intensity chronic exercise demonstrate compromised coupling and disturbance of GSH redox status (23,24). The mitochondrial theory of ROS production is also supported by the training adaptation of mitochondrial antioxidant enzymes. Higuchi et al. (25) show that mitochondrial Mn-SOD activity was induced after endurnace training, whereas cytosolic (CuZn) SOD was unaffected. Mitochondrial GPX shows a greater extent of training adaptation than cytosolic GPX in rat (26). These data provide strong evidence that mitochondria are a major source of ROS production because ROS generated in other cellular locations are unlikely to migrate to mitchondria due to their limited mobility.

The observations that the extent of tissue oxidative damage is proportional to the workload of aerobic exercise support the view that mitochondria are indeed a primary site for ROS generation. During prolonged exercise, energy is supplied primarily by oxidative phosphorylation of ADP via the mitochondrial respiratory chain, which provides a steady source of H_2O_2. Alessio and Goldfarb (27) show that lipid peroxidation measured by thiobarbituric acid reactive substance (TBARS) was correlated with treadmill workload in rats. Also, Kanter et al. (28) report an increased pentane in expired air of human subjects as evidence of tissue lipid peroxidation, and that the pentane concentration is increased proportionally with workload. Furthermore, Ji et al. (29) show that the oxidation of GSH to glutathione disulfide (GSSG) in skeletal muscle increased as a function of workload in rats running on a treadmill. Because oxygen consumption is a function of workload in steady state exercise, these findings suggest that oxidative cell damage may be mediated by enhanced production of ROS from the mitochondria. Finally, in a study attempting to elucidate neutrophil activation during exercise, Pincemail et al. (30) found that the β-adrenorecepter blocker, propranolol (40 mg/kg) significantly suppresses whole body lipid peroxidation induced by exercise in man, using expired pentane as a marker. It is well-known that β-blockade reduces oxygen consumption in myocardial and peripheral tissues during exercise, therefore this study implies that the rate of oxygen consumption in the tissue (primarily in the mitochondria) is the determining factor for ROS production and oxidative stress.

Xanthine Oxidase Theory

Xanthine oxidase (XO)-catalyzed reactions are well-established as one of the major sources of free radical generation in the ischemia and reperfused (I-R) heart (31,32). This notion is based on the findings that, during ischemia, ATP is degraded to ADP and AMP from the energy demand of contracting myocardium. Due to the insufficient oxygen supply, AMP is continuously degraded to hypoxanthine, which may be converted to xanthine and uric acid by XO coupled with the one-electron reduction of O_2, giving rise to $O_2^{\cdot-}$ (33). Figure 2 depicts the role of XO in generating $O_2^{\cdot-}$ in the reperfused organs after a brief interruption of blood supply. To activate this pathway, several conditions must be met.

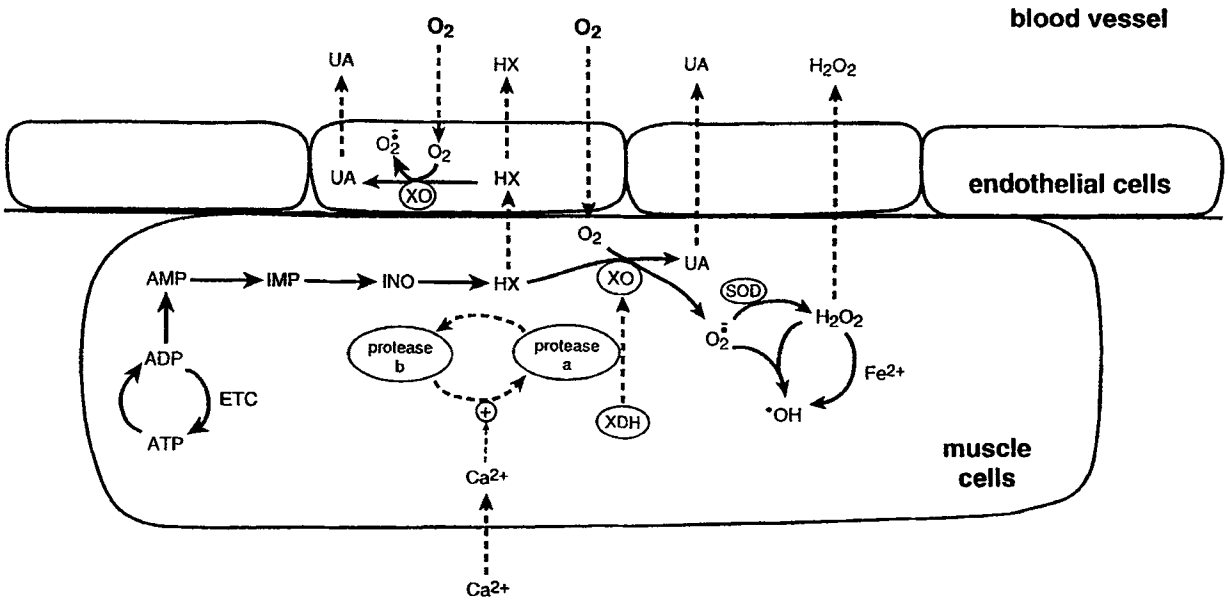

Fig. 2: The role of xanthine oxidase (XO) in free radical generation in the muscle and endothelial cells. ETC, electron transport chain; HX, hypoxanthine; UA, uric acid; XDH, xanthine dehydrogenase. Reactions are not balanced stoichiometrically.

First, sufficient amounts of the substrates hypoxanthine and xanthine must be present in the tissue. Second, the enzyme XO must be present in its active form, (i.e., the oxidized form). The reduced form of XO, xanthine dehydrogenase (XDH), utilizes NAD^+ rather than O_2 as the electron acceptor and does not produce $O_2^{\cdot-}$ (34). XDH is converted to its active form, XO, by intracellular protease, which may be activated by Ca^{2+} or by a redox mechanism of the enzyme (31). Third, O_2 must be available as the electron acceptor. This is why ROS is produced during the reperfusion instead of the ischemic phase.

There is some evidence that high-intensity exercise mimics the situation of heart I-R and may activate the XO pathway (12,35). Hypoxanthine is reported to accumulate after intense muscular contraction as a result of adenine nucleotide degradation (36), and the uric acid concentration is shown to increase in both contracting arm muscle and in the plasma (37). These findings suggest that XO is active, because contribution of other pathways to uric acid production is negligible. Sahlin et al. (38) show that blood hypoxanthine and xanthine concentrations increased dramatically in human subjects after intense exercise. Skeletal muscle was thought to be the source of these purine metabolites resulting from AMP breakdown. Radak et al. (39) show that XO activity is increased 10-fold in the plasma of rats after repeated high-intensity runs to exhaustion and that plasma XO activity is correlated to lactate concentration. The authors propose that the origin of the enzyme was from the endothelial cells of the muscle where XDH was converted to XO via a Ca^{2+}-activated protease. In a follow-up study, the same authors demonstrate that exhaustive exercise also causes an elevation of XO activity, along with GPX activity and TBARS content in rat liver (40). A recent study by Rasanen et al. (41) shows that strenuous exercise in horses increased peroxyl radical production and XO activity in the plasma. Furthermore, uric acid concentration increased exponentially in relation to workload indicating a rapid degradation of purine products.

However, the theory that XO plays a significant role in free radical production during exercise remains tentative. Although hypoxanthine and xanthine tend to accumulate during intense muscle contraction, this probably occurs only during ischemic exercise when blood flow and oxygen supply to muscle are low, or when exercise involves only a small muscle mass such as during arm exercise. Dynamic exercise involving a large muscle mass does not result in an appreciable accumulation of purine nucleotide degradation products (38), because sufficient oxygen supply ensures ATP to be replenished primarily via mitochondrial oxidative phosphorylation. Furthermore, the increased uric acid levels in the blood could have been formed through XDH rather than XO (38). XO activity is low in skeletal muscle, and data from muscle XO activity in response to exercise are currently not available. Whether or not the XO activity detected in the plasma after strenuous exercise comes from skeletal muscle remains to be verified. However, Hellsten et al. (42) recently reported an increase in XO-immunoreactive cells, presumably capillary endothelial and leukocyte cells, in human subjects after 7 days of intense exercise training. Two months postexercise, these subjects showed elevated plasma creatine kinase activity and muscle hydroxyproline content. The authors suggest that XO activation might be etiologically related to muscular oxidative damage during exercise. Thus, the XO theory appears to hold special merit when skeletal muscle undergoes an adenine nucleotide deficit and/or a hypoxic phase followed by reoxygenation.

Neutrophil Theory

Polymorphoneutrophils (PMN) are blood-borne cells that play a critical role in defending tissues from viral and bacterial invasion (43). During the acute phase response, PMN migrate to a potential injury site such as inflammation and release two primary factors for phagocytosis, lysozymes and $O_2^{\cdot-}$. Lysozymes facilitate the breakdown of damaged tissue cells, while $O_2^{\cdot-}$ is produced by melyoperoxidase and NADPH oxidase (43). This is especially important if $O_2^{\cdot-}$ is involved in the initial oxidative injury because, $O_2^{\cdot-}$ activates a chemotactic factor that attracts neutrophils (44). Figure 3 shows the process of neutrophil activation and ROS production during an acute phase response. Although there has been increasing research in the past decade with respect to exercise and immune function, it was only recently that the links between neutrophil activation and ROS production started to emerge (3,43-45). Some researchers propose that the body's immune system responds to an acute bout of intense exercise in much

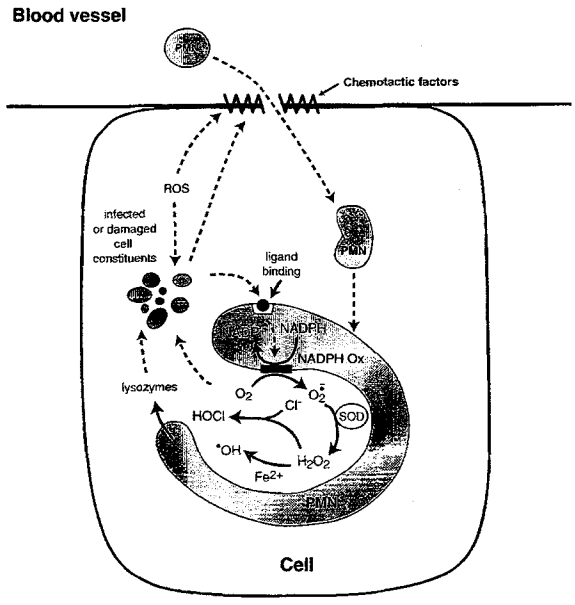

Fig. 3: The process of polymorphoneutrophil (PMN) infiltration and activation in the cell. NADPH Ox, NADPH oxidase; ROS, reactive oxygen species; SOD, superoxide dismutase. Reactions are not balanced stoichiometrically.

the same way as to sepsis wherein they share a common mediator, ROS (46).

It has long been recognized that strenuous exercise can elicit muscle injury accompanied by an inflammatory response, which is characterized by increased protease and lysozymal enzyme activities in working muscle (10). The response can last from several hours to several days after the cessation of exercise, depending on the intensity and duration of exercise. Furthermore, it was discovered that the biomarkers of the inflammatory responses often coincided with elevation of antioxidant enzyme activities such as GPX and CAT (10). These findings have prompted some investigators to hypothesize that ROS might be produced in post-exercise tissues possibly caused by inflammation and neutrophil invasion (8). While this inflammatory response is considered critical in removing damaged proteins and preventing bacterial and viral infection, ROS released from neutrophils can also cause secondary damage, such as lipid peroxidation (8). Hack et al. (47) show that an acute bout of exhaustive exercise in human significantly increased cell counts of leukocyte, lymphocytes, and neutrophils. Phagocytosis assays reveal that ingestion capacity was elevated immediately after exercise up to 24 hr post-exercise, whereas a significant increase in $O_2^{\cdot-}$ production was noticed only at 24 hr post-exercise. Additional evidence for neutrophil involvement in ROS generation comes from studies of eccentric exercise. Meydani et al. (45) show that following an acute bout of eccentric exercise in sedentary men, circulating cytokine (interleukin-1) levels are significantly elevated, possibly released from activated monocytes. Interleukin-1 has been shown to be induced by $O_2^{\cdot-}$ in vitro (3). Furthermore, vitamin E administration attenuated the urinary markers of lipid peroxidation found during the post-exercise period, verifying the oxidative nature of the injury (45). Recently, Smith et al. (48) reported that one hour of moderate exercise increased neutrophil H_2O_2 generation by three-fold under in vitro challenge as well as receptor expression. However, current data does not elucidate a clear role for neutrophils in enhancing ROS production during exercise. Given the time required for neutrophil infiltration, this pathway probably is not the primary source of free radical production and oxidative stress during short-term exercise. However, it may serve as an important secondary source of free radical production during the recovery period following heavy exercise. Particularly, it may contribute to oxidative tissue damage during ultra endurance exertion, such as marathon running, or to injury observed after eccentric exercise.

Other ROS Generating Pathways

Under physiological conditions, liver microsomes generate oxygen free radicals primarily via the cytochromes P_{450} system (4,6). NADPH is oxidized with the catalysis of the mixed function oxidase, giving rise to $O_2^{\cdot-}$, which can be dismuted to H_2O_2. It is known that the rate of H_2O_2 production is increased at elevated oxygen consumption in the microsome (1). Whether or not this pathway contributes to overall ROS production during exercise is unknown; however, habitual exercise has been shown to modify the amount of ROS production in liver microsomes. Kim et al. (49) report that oxidation of DCF, a detector of intracellular ROS production, is increased in the liver microsomes from old rats, and that chronically active animals produce less ROS than their sedentary counterparts. Exercise is also likely to alter ROS production by certain pro-oxidant drugs via this pathway, because hepatic blood flow is decreased during exercise, causing changes in the pharmacokinetics and pharmacodynamics of drug metabolism (50).

The heart releases norepinephrine from sympathetic nerve endings under various stress conditions, including heavy exercise. Circulating catecholamine levels are also increased during prolonged exercise. Catecholamines enhance myocardial and skeletal muscle oxidative metabolism via activation of β-adrenergic receptors, thereby potentially increasing ROS production via a mitochondrial pathway. Furthermore, autoxidation of adrenaline to adrenochrome is associated with $O_2^{\cdot-}$ formation, which has been identified as a possible source of ROS production in heart I-R injury (31). β-Blockade is shown to reduce oxidative stress markers in plasma of human subjects working at high intensity (30). However, the quantitative significance of catecholamine as a source of ROS production during exercise has not been investigated and remains unclear.

Peroxisomes are organelles in the cell involved in non-mitochondrial oxidation of fatty acids and D-amino acids. Under physiological conditions peroxisomes contribute to the steady state production of H_2O_2 but not $O_2^{\cdot-}$ (6). At rest, liver is the primary organ where peroxisome contribution to H_2O_2 production is important. Prolonged starvation has been shown to increase H_2O_2 generation mainly because of the increased fatty acid oxidation in this organelle (51). Similar to the situation of starvation, fatty acids are the primary energy substrate for the myocardium and skeletal muscle during prolonged exercise; therefore, peroxisomes are potential sites for ROS production. The findings that CAT activity is increased after an acute bout of exercise in muscle seem to support this hypothesis (27,29). Leeuwenburgh and Ji (52) show that 48-hr starvation in rats significantly suppresses the hepatic GSH:GSSG ratio, accompanied by an increased MDA content in liver and skeletal muscle. Unfortunately, CAT activity was not measured in this study to evaluate the potential involvement of peroxisomes.

Regulation and Adaptation of Antioxidant Systems

Although oxygen free radicals are generated as natural byproducts of various biological pathways, serious cell and tissue damage does not usually occur under physiological conditions. Aging might be an exception, during which the slow but the deleterious effect of ROS leads organisms to functional deterioration and death (5).

Even heavy physical exercise rarely causes large scale oxidative damage in healthy individuals. This is because higher organisms have developed a remarkably efficient antioxidant system during the course of evolution (1). Thus, the extent of oxidative damage during physical exercise is determined not only by the level of free radical generation, but also by the defense capacity of antioxidants. Available data suggest that each of the antioxidant systems may have a different response to acute and chronic exercise depending upon their biochemical and molecular mechanism of regulation (53). In recent years, there is a general awareness of the importance of antioxidants, but insufficient knowledge often leads to the false conclusion that physically active people need antioxidant supplementation. The following section intends to clarify the specific role of each category of antioxidants during acute and chronic exercise, so that the necessity of supplementation and/or dietary manipulation of antioxidants can be discerned.

Non-Enzymatic Antioxidants

This category of antioxidants refers to antioxidant vitamins (vitamin E, vitamin C, and β-carotene), GSH, and other thiols. Several biological compounds such as α-lipoic acid, uric acid, and ubiquinone also demonstrate antioxidant functions, but they are not considered vitamins as most mammals can synthesize these compounds and their functions are not exclusively restricted to antioxidants (4,54).

Vitamin E (α-tocopherol) is the most well-known, lipid-soluble free radical scavenger. Its unique location in the cell membrane enhances its efficiency to quench free radicals originating from the mitochondrial inner membrane and other biomembranes (55). Vitamin E is essential for normal cell function during exercise, and is best illustrated in studies where animals are depleted of tissue vitamin E by feeding a vitamin E-deficient diet beginning at the early stage of life. Davies et al. (15) found that vitamin E deficiency exacerbates muscle and liver free radical production and enhances lipid peroxidation and mitochondrial dysfunction in the exhaustively exercised rats. Endurance performance is also reported to decrease in rats fed vitamin E-deficient diets (15,56). Also, vitamin E deficiency is shown to enhance lipid peroxidation, disturb GSH/GSSG redox status, and cause early fatigue in the diaphragm muscle during resistance breathing in rats (57).

Skeletal muscle contains approximately 30-50 nmol of vitamin E per g of wet weight, with considerable differences between muscle fiber types, whereas the concentration of vitamin E in the heart and liver amounts to 60-70 nmol/g (58,59). While an acute bout of exercise does not seem to significantly affect vitamin E content in tissues, its concentration is shown to decrease in a number of tissues, such as skeletal muscle, liver, and heart, in rats after endurance training (59-61). More dramatic changes are observed when tissue vitamin E levels are expressed per unit of mitochondrial ubiquinone content (58). The reduction of tissue vitamin E after training probably reflects the increased free radicals production at the mitochondrial inner membrane during exercise, which gradually depletes vitamin E reserves.

Dietary supplementation of vitamin E may increase tissue resistance to exercise-induced lipid peroxidation. Kanter et al. (28) show that daily supplementation with a vitamin mixture containing 600 mg α-tocopherol for 6 weeks significantly decreased levels of serum MDA and expired pentane, both at rest and after 30 min of treadmill exercise at 60 and 90% VO_2max. Goldfarb et al. (62) report that rats supplemented with a 250 IU vitamin E/kg diet for 5 weeks have lower TBARS and lipid peroxide levels in plasma and leg muscles after one hour treadmill exercise compared to controls. Sumida et al. (63) also demonstrate a protective effect with vitamin E supplementation (300 mg/day) in reducing serum MDA concentration and enzyme markers of tissue damage during exercise. Kumar et al. (17) show that dietary supplementation with vitamin E for 60 days completely abolished the exercise-induced free radical production and lipid peroxidation in rat myocardium.

These findings support the recommendation by Packer (55) that humans involved in an active lifestyle consider increasing daily dietary vitamin E intake. However, despite the aforementioned beneficial effects, no study has yet demonstrated improved physical performance as a result of vitamin E supplementation (13,64).

Vitamin C (ascorbic acid) is a water-soluble antioxidant existing in the cytosol and extracellular fluid. Its chemical properties allow it to interact directly with $O_2^{\cdot}$ and $^{\cdot}OH$, thereby functions as an antioxidant (65). It can also regenerate oxidized vitamin E, wherein ascorbate is oxidized to dihydroascorbate (DHA). DHA may be reduced by a GSH and/or dihydrolipoic acid redox cycle (1,66). Vitamin C is especially efficient in scavenging free radicals formed in the aqueous phase such as plasma, thereby preventing damage to erythrocyte membrane (65). The importance of vitamin C in protecting against exercise-induced oxidative stress is not well-established, partly because most mammalian species synthesize vitamin C, making a deficiency study rather difficult. Vitamin C also performs numerous functions that are not related to those of antioxidants (67). By reducing dietary vitamin C content to 10% of the normal values (0.2 g/kg), Packer et al. (68) demonstrated that myocardial capacity to oxidize pyruvate, 2-oxoglutarate and succinate was significantly reduced in guinea pigs (which can not synthesize vitamin C) vs. controls. As a result, running time to exhaustion was significantly shortened by dietary vitamin C deficiency.

It is well-known that given at high doses, vitamin C can behave as a pro-oxidant (4). This is because ascorbate reacts with transition metal irons to form ROS, including $^{\cdot}OH$ (1,4). Thus, it is interesting to note that in the aforementioned study, a group of guinea pigs supplemented with twice the normal amount of vitamin C in the diet also exhibited similar metabolic defects in the heart and early fatigue during prolonged exercise,

possibly from the oxidative stress caused by excessive vitamin C. Since one of the primary antioxidant functions of vitamin C is to recycle vitamin E, Gohil et al. (56) investigated the effect of dietary vitamin C supplementation on vitamin E deficiency during training in rats. They found that vitamin C could not prevent a decrease of endurance time and some tissue-specific disorders of mitochondrial function caused by vitamin E deficiency.

The effect of dietary supplementation of vitamin C has also been studied in human subjects involved in physical exercise (64). Although large doses of vitamin C intake was claimed to reduce fatigue and muscle damage in several studies, no specific oxidative stress markers were measured; therefore, it is difficult to determine whether the observed benefits were related to the antioxidant functions of vitamin C.

Glutathione GSH (γ-glutamylcysteinylglycine) is the most abundant non-protein thiol source in the cell and its concentrations in most tissues are in the millimolar range. GSH serves multiple functions in protecting tissues from oxidative damage and in keeping the intracellular environment in the reduced state (69). GSH reduces hydrogen and organic peroxides via a reaction catalyzed by GPX; it serves as a scavenger of $^{\bullet}OH$ and singlet oxygen ($^1O_2$); and, GSH is believed to reduce tocopherol radicals, either directly, or indirectly by reducing DHA radical, thereby prevent lipid peroxidation (66). By donating its proton, GSH is oxidized to GSSG, which can be reduced back to GSH by glutathione reductase (GR), a flavon-containing enzyme, using NADPH as the reducing power.

GSH can be synthesized from endogenous or dietary amino acids, but only the liver contributes to significant *de novo* GSH synthesis, supplying 90% of the circulating GSH (69). Insulin stimulates hepatic GSH synthesis, while glucagon and vasopressin promote the efflux of GSH to the plasma (70). During prolonged exercise, plasma insulin concentration is suppressed, whereas concentrations of glucagon and vasopressin are increased, creating a hormonal milieu in favor of hepatic GSH export. Indeed, a decreased liver GSH content after exercise has been consistently reported in rodent studies (71-73). Although part of the GSH decline could possibly be explained by increased oxidation, the fact that liver GSSG was found to be either unchanged (74) or decreased (73) after exercise indicates that the majority of the GSH decrease was not due to oxidation but efflux. A possible release of GSSG into the bile under oxidative stress cannot be ruled out, however, Villa et al. (75) show that biliary GSSG release is suppressed during heavy exercise. In contrast to the liver, skeletal muscles seem to import GSH from the plasma during exercise despite the concurrent oxidation of GSH to GSSG (29,73,74,76). GSH and total glutathione (GSH+GSSG) content in the oxidative types of rat hindlimb muscles are found to increase significantly after an acute bout of exercise, and the magnitude of increase appears to depend on exercise intensity (29). As a result, there is little change in the GSH/GSSG ratio after exercise and only a modest decrease of the GSH/GSSG ratio occurrs at exhaustion (74). Further support for a possible GSH uptake by non-hepatic tissues was provided in the finding that cysteine and glutamate concentrations are significantly increased in the exercising muscle (29,74). However, muscle GSH content may be eventually decreased when the hepatic GSH reserve is diminished and GSH utilization exceeds GSH uptake during prolonged exhaustive exercise (72,77). Similar to the skeletal muscle, the heart is capable of importing and utilizing GSH to cope with the increased ROS production. This is supported by the finding that heart γ-glutamyl transpeptidase (GGT) activity was upregulated in mice after an acute bout of swimming (78). However, myocardial GSH did not increase, but instead decreased during an acute bout of exercise (78). The different responses of muscle and heart GSH to acute exercise may be explained by the different activities of GGT, the enzyme which initiates a series of steps for tissue GSH uptake (73,79). Plasma GSH appears to be relatively stable during prolonged exercise at moderate intensity (73), but during heavy exercise such as marathon running, hepatic GSH output may not be sufficient to match the increased utilization by the erythrocytes and extrahepatic tissues, causing a net oxidation and decline in blood (80).

Chronic exercise training at high intensity is shown to increase GSH content in selected leg muscles of dogs (72) and in the deep portion of the vastus lateralis muscle (DVL) of rats (79,81). Myocardial GSH levels have also been reported to increase significantly in rats after swim training (82,83). The mechanism for such adaptation is still unclear. Marin et al. (84) demonstrate in dog muscle, that after a year-long training program, activities of GGT and γ-glutamylcysteine synthetase (GCS) were increased, which seems to explain the training-induced GSH content. However, in a recent study, we reported an elevation of GSH level in rat DVL muscle after training without a concomitant increase in GGT or GCS activity (79). Furthermore, we have found a decreased GSH content in rat soleus muscle after training (79,81). Because tissues are capable of exporting GSSG under oxidative stress (85), if the rate of GSH consumption exceeds that of import during exercise, a tissue may show a net deficit of GSH.

GSH deficiency is associated with a wide range of physiological and biochemical disorders (69). Tissue GSH may be depleted with the administration of either a GSH conjugate, such as diethylmaleate (DEM), or a specific inhibitor of GCS, buthionine sulfoximine (BSO). Thus, experimental GSH depletion is expected to exacerbate exercise-induced oxidative stress in the various tissues. Morales et al. (86) show that DEM administration (i.p.) could cause a marked reduction of contractile properties of rat diaphragm muscle undergoing resistant breathing; whereas DEM per se had no significant effect. Sen et al. (72) report that rats treated with BSO twice-a-day for 4 days decreased endurance performance during treadmill running, and this was associ-

ated with increased GSSG/GSH ratio and TBARS concentration in several types of skeletal muscle and heart. Using a 12-day BSO treatment regimen in mice, Leeuwenburgh and Ji (73) show that severe GSH depletion results in a significant down-regulation of liver GPX activity and muscle GGT activity. Also, a striking decrease of mitochondrial enzyme citrate synthase activity is observed in liver, kidney, and skeletal muscle. Exhaustive swimming in GSH-depleted mice enhances liver and muscle MDA formation, but no loss of endurance is found. The mild oxidative damage observed in this study is probably due to an adaptation of mice to chronic BSO treatment, evidenced by increased muscle GPX activity and liver GR and GST activities.

Because exercise disturbs the GSH-GSSG redox status and diminishes total GSH reserve in the body, it is conceivable that GSH supplementation during acute and chronic exercise might be beneficial for the maintenance of GSH homeostasis. Novelli et al. (87) show that both acute GSH injection (i.p.) at the dosages of 250-1000 mg/kg body and chronic GSH supplementation at 250 mg/kg for 7 days doubled swimming endurance times in mice. However, total swimming time in that study was only 2-3 minutes and tissue GSH levels were not measured; it is therefore difficult to evaluate the contribution of GSH to endurance performance. In our laboratory, acute GSH supplementation (i.p.) in mice was found to enhance endurance performance by more than 50% (6 hr vs. 4 hr) with no change in tissue GSH levels (88). GSH supplementation is complicated by the inability of tissues to take up plasma GSH due to the strong negative feedback inhibition of GSH exerted on GCS (89). This is demonstrated by the observation that plasma GSH was elevated by 20-fold as a result of a single intraperitoneal injection of GSH; whereas, GSH concentration in the liver and other tissues showed little change (72,88,90). The difficulty in raising tissue GSH levels appears to hinge on the delivery of cysteine across the cell membrane. Thus, several compounds that serve as cysteine precursors have been used as alternatives to GSH, such as N-acetylcysteine (NAC), 2-oxy-4-thiazolidine-carboxylic acid (OTC), and GSH ethyl ester (91,92). Oral NAC supplementation in human subjects for two days (total dose 2.4 g) has been reported to decrease GSSG levels and blood lipid peroxidation indices provoked by an acute bout of exercise (93). Recently, Reid et al. (20) showed that NAC administration improved muscle contractile functions and reduced low-frequency fatique in humans. Regardless of the mechanisms in the mentioned studies, supplementation of exogenous thiol sources has shown some clear merit in reducing oxidative stress during exercise (7).

Ubiquinone (Q_{10}) As an electron carrier, ubiquinone is rich in the mitochondrial inner membrane. An early study by Gohil et al. (58) showed that training could significantly increase ubiquinone content in skeletal muscle and adipose tissues. Reduced ubiquinone acts as an antioxidant in vitro and its role as an antioxidant in vivo has been proposed (94). Tissue slices from rats fed a high-ubiquinone diet demonstrated more resistance to hydroperoxide-induced lipid peroxidation than those from rats fed a control diet (95). These antioxidative properties have prompted several studies using dietary supplementation of Q_{10} to evaluate its protective function during exercise. For example, Shimomura et al. (96) report that Q_{10} administration attenuated muscle creatine kinase and lactate dehydrogenase release in rats caused by down-hill running. However, these studies did not clearly establish the role of Q_{10} as an antioxidant in vivo. Furthermore, little data is available regarding the interaction of Q_{10} with other antioxidants during exercise.

Uric acid Uric acid is the end product of purine metabolism, appearing in high concentrations in the circulation after heavy muscular contraction and in the effluent of ischemia-reperfused organs (12). This change results from an insufficient intramuscular ATP supply causing excessive adenine nucleotide degradation and accumulation of hypoxanthine and xanthine (36-38). These purine metabolites are released from the muscle into the blood and a portion of these compounds presumably is converted to uric acid by XO located in the endothelial cells of the blood vessels (Figure 2). Uric acid's function as a potential antioxidant has been recently emphasized (4). Besides being an excellent scavenger of $^{\bullet}OH$, uric acid may preserve plasma ascorbic acid under oxidative stress (97). Because an acute bout of exercise has been shown to increase blood uric acid concentrations in human subjects (38,98), it is not unreasonable to speculate that the increased uric acid may serve as one of the protectants against blood-borne sources of ROS, thus reducing oxidative stress to erythrocytes and other tissues. However, there is currently no data to substantiate the antioxidant function of uric acid in exercise.

α-Lipoic acid The function of α-lipoic acid in the catalytic mechanism of oxidative decarboxylation by pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, and branched-chain ketoacid dehydrogenase is well understood. Recently, a great deal of attention has been given to the antioxidant potential of its reduced form, dihydrolipoic acid (DHLA). Both α-lipoic acid and DHLA have exhibited specific scavenging capacities for a variety of free radicals, such as $O_2^{\bullet}$, $^{\bullet}OH$, $^1O_2$, peroxyl radical, and hypochlorous radical (54). They are chelators of transition metal ions, and therefore prevent damaging free radical chain reactions. DHLA is capable of regenerating other antioxidants such as vitamin E and vitamin C from their radical forms either directly or indirectly via the GSH-GSSG redox cycle. Thus, DHLA prevents vitamins E and C deficiencies possibly through increasing intracellular GSH levels (54). Perhaps the most intriguing and complex biological function of DHLA is its proposed effect on antioxidant gene expression via the regulation of the nuclear factor, NF-κB. DHLA has

been shown to influence both the dissociation of the inhibitory subunit IκB from NF-κB complex and the binding of the activated NF-κB (p50 and p65) to DNA (for a brief summary, readers are referred to the next section). The overall effect could be either stimulatory or inhibitory depending on the redox state of the cell and the relative concentrations of α-lipoic acid and DHLA (14,54).

Antioxidant Enzymes

Cells are equipped with a host of enzymes that are directly or indirectly involved in the antioxidant defense against ROS. Enzymes that provide primary defenses include SOD, CAT, and GPX. GR and enzymes producing NADPH, such as G6PDH, malic enzyme, and isocitrate dehydrogenase are important in reducing GSSG to GSH, such that an adequate substrate level is maintained for GPX. GST is an important enzyme in metabolizing pro-oxidant xenobiotics in the liver. Secondary defense includes a group of loosely defined enzymes, which either repair cellular damage caused by ROS or remove the damaged molecules, such as phospholipase A_2 or specific proteases (4). Cytochrome c oxidase also has an indirect antioxidant function because of its extraordinary affinity with O_2, thereby decreasing the probability of O_2 to undergo one-electron reduction in the mitochondrial inner membrane (6). The catalytic mechanisms and the regulation of various antioxidant enzymes have been reviewed extensively by previous authors (1,4,6,11,13). Thus, the current review focuses primarily on the effects of acute and chronic exercise on the various primary antioxidant enzymes.

Superoxide Dismutase An acute bout of exercise has been shown to increase SOD activity in a number of biological tissues including heart (21,78,99), liver (26,43,74,100,101), blood platelets (102), erythrocytes (103), and skeletal muscle (29,74,99,103-105). However, reports on the effect of exercise on SOD varies greatly among different studies with many showing no significant effect (see 105 for a review). Exercise intensity seems to play an important role in determining SOD response to acute exercise, because increased SOD activity has been reported in most studies involving exhaustive exercise in either animals or human, whereas exercise with moderate intensity or shorter duration generally has shown no effect (105). Furthermore, CuZn-SOD appears more likely to be activated by an acute bout of exercise than Mn-SOD (105,106). Because SOD can be activated by partial occupancy of the enzyme by O_2 in vitro (6), it is proposed that the increased SOD activity may be an indication that O_2 production is increased during exercise (106). However, the role of O_2 in SOD activation during exercise is uncertain, because O_2 has a very short half-life and limited diffusibility (1,6). Furthermore, most SOD assays involve an in vitro O_2 generating system making it impossible to evaluate the influence of O_2 binding on enzyme activation. SOD also demonstrates an unusual enzyme kinetics whereby its activity is increased almost indefinitely with increased substrate concentration and no apparent Km or Vmax (1,6). There is some controversy as to whether high SOD activity is desirable, because the dismutation product, H_2O_2, has a higher diffuseability and a longer half-life, and may become a source of $^{\bullet}OH$ production (107). However, O_2 can generate H_2O_2 independent of SOD in the cell by attacking the ion-sulfur protein (4Fe-4S) cluster, thereby releasing Fe(II), which sets the stage for $^{\bullet}OH$ production via the Fenton reaction or via Haber-Weiss reaction (1). This "cooperativity" between O_2 and H_2O_2 is an important mechanism for cellular damage, particularly DNA damage (107). In this view, an elevation of SOD in response to heavy physical exercise is probably not only desirable, but vital, given the proximity of O_2 generation to mitochondrial DNA. Radak et al. (39) show that down-regulation of Mn-SOD by high-altitude exposure is associated with an increased lipid peroxidation in skeletal muscle, whereas supplementation with an SOD derivative (SM-SOD) attenuated plasma lipid peroxidation and activation of XO in rats that were run to exhaustion.

The effect of chronic exercise training on SOD activity has shown great variability (105). Some studies have revealed no significant change in SOD activity with training; however, increased SOD activity was reported by other investigators in the liver (108), locomotive muscles (25,79,81,109,110), heart (17,111), and diaphragm (112). Compared to GPX and CAT, SOD demonstrates a relatively small inter-tissue difference even though these tissues vary greatly in their oxidative and anti-oxidative potentials (13). Because SOD activity can be increased with increased O_2 production (non-saturating kinetics), in most tissues there may be sufficient SOD activity to convert O_2 to H_2O_2, (i.e., dismutation of O_2 may not be rate-limiting in ROS removal). Nevertheless, rigorous training is shown to increase SOD activity even in post-mitotic tissues such as the myocardium (17,111). In addition to enzyme activity, training is also shown to increase SOD protein concentration in rat skeletal muscle (105). We have recently investigated the enzyme activity and mRNA abundance in both CuZn-SOD and Mn-SOD in the heart, liver, and three types of skeletal muscles in response to endurance training (79,113). There was a 125% increase in CuZn-SOD mRNA abundance in DVL muscle, associated with a 35% elevation of CuZn-SOD activity, with training. The mRNA abundance of CuZn-SOD in the liver and heart also increased significantly, but there was no accompanying increase in enzyme activity. This finding contrasts with Higuchi et al. (25) who show that only Mn-SOD, not CuZn-SOD, is induced by training. Thus, in response to training, the two SOD isozymes seem to be regulated by different mechanisms.

Glutathione Peroxidase Reports on the effect of an acute bout of exercise on GPX activity in various tissues are inconsistent in the literature. Several previous stud-

ies show no change in this enzyme in skeletal muscle after acute exercise (114,115), whereas others report that GPX activity in muscle is elevated following intense exercise (29,52,74,104,109). Furthermore, heart (109) and platelet (102) GPX activity is shown to be elevated after exercise. The mechanism by which exercise activates GPX is not clear, presumably the activation reflects an increased production of intracellular H_2O_2 and lipid hydroperoxide (13,106). GPX is a selenium-containing enzyme and demonstrates an unique kinetics with an infinite Vmax and Km. Therefore, saturating conditions for both of GPX's substrates, hydroperoxide and GSH, cannot be reached (116). The kinetic properties of this enzyme imply that, at an adequate GSH concentration (millimolar range), the activity of GPX is mainly determined by its cosubstrate, hydroperoxide. However, when tissue GSH is depleted by BSO treatment, GPX activity shows a significant reduction in the liver and heart (73). This is probably explained by the fact that GPX is susceptible to inactivation by O_2 and hydroperoxides in vitro due to the oxidation of the selenocysteine residue at the enzyme's active site (117). A high concentration of GSH is required to keep this active site in the reduced state. Recently, we studied the effect of an acute bout of exhaustive exercise on the relative abundance of GPX mRNA in a variety of tissues. While GPX activity was found significantly increased, by as much as 40%, in the DVL muscle (113), no change in GPX mRNA levels was observed, possibly indicating that the increased enzyme activity in response to exercise is mediated by a translational or post-translational mechanism (unpublished data).

Most studies show that GPX activity is inducible by chronic exercise training in skeletal muscle, including the diaphragm (22,26,79,81,110,112,118). However, GPX adaptation to training appears highly muscle fiber-specific. Only type 2a muscles, represented by the DVL and the red gastrocnemius muscles, have consistently shown an elevated GPX activity after endurance training. Furthermore, the magnitude of GPX training adaptation seems to depend upon exercise duration rather than exercise intensity (110). Moreover, endurance training is shown to result in a greater increase in the mitochondrial fraction of GPX activity than the cytosolic fraction (26). In the diaphragm, GPX upregulation is greater in the costal vs. crural portion, possibly reflecting the different muscle fiber type and workload between the two regions (112). Although the critical role of selenium in GPX training adaptation is well-established (26,101), little is known about the gene regulation of GPX in response to chronic exercise training. Preliminary studies conducted in our laboratory reveal that training elicits no significant alteration in the relative abundance of GPX mRNA in DVL muscle, although a prominent increase in GPX enzyme activity with training was observed (113). The discrepancy between GPX activity and mRNA data suggest that the enzyme may be regulated by translational and/or post-translational mechanisms.

Catalase With only a few exceptions (29,74,119,120), most studies report no significant alteration in CAT activity with acute exercise or chronic training (8,13). With a catalytic mechanism similar to SOD, CAT activity might be expected to increase during exercise due to increased H_2O_2 production. However, CAT is located primarily in the peroxisome, whereas the main source of H_2O_2 is mitochondria (6). Recently, Luhtala et al. (121) reported that the submitochondrial particles of rat liver contain a significant fraction of CAT activity and that it dramatically increases during aging, whereas the cytosolic fraction shows no significant alteration. It would be interesting to investigate whether this mitochondrial CAT can be altered by acute exercise or chronic training. It is important to recognize that although GPX and CAT have an overlap of substrate H_2O_2, GPX (at least in mammals) has a much greater affinity for H_2O_2 at low concentrations (Km = 1 μM) than CAT (Km = 1 mM) (122). Thus, the threshold for activation for CAT may be higher than for GPX. These kinetic properties probably explain why most studies find no significant alteration in CAT activity in several tissues that show prominent training adaptation of GPX (11,13).

Glutathione reductase Although GR is not directly involved in removing ROS, it serves an important role in converting GSSG to GSH, thereby maintaining GPX catalytic function and a reduced intracellular redox status (1). In most tissues, GR activity is far lower than GPX activity, and this, at least in part, accounts for an elevation of GSSG during prolonged exercise (7). GR activity has been shown to increase in rat skeletal muscle after an acute bout of exercise along with increased GPX activity (29,74). Also, erythrocyte GR activity was reported elevated after prolonged exercise in humans (123,124). It is interesting to note that GSH depletion with BSO treatment resulted in a 2.5-fold increase in GR activity in mouse liver, whereas GPX activity was severely hampered (73). This finding suggests that there might be a coordinated regulation of GPX and GR in response to oxidative stress.

Glutathione sulfur-transferase GST catalyzes the conjugation of GSH with a wide variety of organic compounds, including certain species of hydroperoxide, thereby shares peroxidase activity with GPX (125). Unlike GPX, GST activity is not affected by selenium deficiency, however, adequate GSH concentration is critical for the enzyme's catalytic function. GST a, b, and c have a quite low Km for GSH (0.1-0.2 mM), whereas GST e has a Km of 2.0 mM (125). Recently, we reported that chronic BSO treatment increased GST activity (using 1,2-dichloro-4-nitrobenzene as substrate reflecting GST a, b, and c activity) in mouse liver and kidney; on the other hand, muscle and heart GST activities down regulated in response to GSH depletion (73). The increased hepatic GST activity was probably a compensation for the diminished GPX activity. Acute and chronic exercise seem to have minimal effects on GST activity in skeletal muscle (13). However, erythrocyte GST activity is shown to increase after an acute bout of

prolonged exercise (123), and liver GST is reportedly induced by endurance training (72,126). Reddy et al. (127) recently showed that 4 months of swim training significantly increased hepatic GST activity in rats and that the induction was caused by an upregulation of certain GST subunits, particularly Ya_1 (4-fold), which displays the highest peroxidase activity.

Summary of Findings

The mechanism by which antioxidant enzymes can be upregulated in response to physical exercise within a relatively short period is largely unknown. There is still insufficient knowledge about either the kinetic properties or molecular regulation of these enzymes in mammalian tissues (53). In the past decade an intensive search has been conducted for the regulatory mechanism of gene expression for antioxidant enzymes in response to oxidative stress. The following is a brief summary of the major findings that may be important for the understanding of exercise-associated alterations of these enzymes. (i) In the prokaryotes, two ROS-responsive transcription factor systems have been identified (i.e., oxyR and soxR). They control the gene expression of antioxidant enzymes responsible for the removal of H_2O_2 and O_2, respectively (128). (ii) Antioxidant enzyme regulation in eukaryotic cells is poorly understood, however, AP-1 and NF-κB are two transcription factors that play an important role and that are regulated by the redox state of the cell (129). (iii) NF-κB is activated by a variety of pro-oxidants, including ROS (130). Upon stimulation, the inhibitory subunit IκB previously bound to the NF-κB complex dissociates from the two main subunits p50 and p65, allowing them to translocate into the nucleus. (iv) Once in the nucleus, p50 and p65 may serve as inducers of transcription for antioxidant enzymes. (v) Finally, the activation of NF-κB is inhibited by treatment of antioxidants. These advances in knowledge about antioxidant gene regulation have expanded the horizon of research in free radical chemistry and exercise, and await vigorous investigation in the years to come.

CONCLUSION

There is both direct and indirect evidence that heavy physical exercise enhances free radical production in skeletal muscle and other tissues. Although increased oxygen flux through mitochondrial electron transport chain is probably the main source for free radical generation, other pathways may also be involved under specific physiological conditions and in specific tissues. Thus, XO may be activated when exercise is performed in conjunction with hypoxia-reoxygenation such as ischemic contraction and high-intensity sprinting. Activation of polymorphoneutrophil provides an additional source of ROS when exercise causes initial tissue damage (whether or not oxidative in nature) and/or triggers an acute phase response of the immune system. Furthermore, these free radical generating mechanisms are not mutually exclusive, therefore oxidative injury may escalate during and after an acute bout of strenuous exercise. The overall consequences of the exercise-mediated free radical generation are still being investigated.

Acute and chronic exercise significantly impacts on the various antioxidant systems in the cell. Activation of antioxidant enzymes and the GSH-GSSG redox cycle during acute exercise can be viewed as a defensive mechanism in response to increased free radical production. However, prolonged heavy exercise causes a transient decline in tissue vitamin E and glutathione reserve. Depletion of an antioxidant nutrient caused by dietary deficiency or pharmacological manipulation can severely hamper the related antioxidant system and exacerbate exercise-induced oxidative stress and tissue damage. Chronic exercise training seems to have dual effects: it induces antioxidant enzymes and stimulates GSH synthesis, however, it also increases the consumption of vitamin E and perhaps GSH, therefore reducing their concentrations in certain tissues.

In evaluating the impact of exercise on the various antioxidant systems, one must keep in mind that many antioxidants have overlapping protective functions that both interact and compensate one another. Further, some antioxidants, such as vitamin C, can exhibit pro-oxidant properties when given in high doses. The interrelationship of antioxidants during exercise is elegantly reviewed by Witt et al. (131). Thus, while supplementation of certain antioxidant nutrients may be beneficial to physically active individuals, a balanced antioxidant status is probably more important than any single antioxidant in protecting against exercise-induced oxidative damage.

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